

Seroprevalence of toxoplasmosis, brucellosis and listeriosis in horses in Hakkari, eastern region of Turkey

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SUMMARY

The aim of this study was to detect the seroprevalences of Toxoplasmosis, brucellosis and listeriosis in horses from the Hakkari city, eastern region of Turkey. For this purpose, serum samples from 74 local horses were obtained and tested using IHA and Sabin-Feldman Dye tests (SFDT) for toxoplasmosis, Serum Tube Agglutination test (SAT) for brucellosis and Osebold Agglutination Test (OAT) for listeriosis. The toxoplasmosis seroprevalences detected with IHAT and with SFDT were 13.5% and 28.3% respectively, the SFDT presenting a higher sensitivity. The seroprevalence of brucellosis was low in horses (9.5%) while a high seroprevalence of listeriosis (48.6%) was evidenced. No significant association between age or sex and toxoplasmosis or listeriosis infections was observed. By contrast, brucellosis seroprevalence was significantly higher in females than in males. These results suggest that horses from the Hakkari region can be considered as potential reservoirs for these infectious agents for other species and may contribute to the disease spreading and to direct (brucellosis, listeriosis) and indirect (toxoplasmosis) contamination of humans.

Keywords: Toxoplasmosis, brucellosis, listeriosis, horse, Hakkari, seroprevalence.

RÉSUMÉ

Séroprévalence de la toxoplasmose, de la brucellose et de la listériose chez les chevaux en Hakkari, Est de la Turquie

L'objectif de cette étude est d'établir la séroprévalence de la toxoplasmose, de la brucellose et de la listériose chez les chevaux de la cité d'Hakkari, région Est de la Turquie. Pour cela, les sérums de 74 chevaux locaux ont été testés par les tests d'inhibition de l'hémagglutination (IHAT) et de coloration au Sabin-Feldman (SFDT) pour la recherche d'anticorps anti-*Toxoplasma*, d'agglutination en tube (SAT) pour détecter les anticorps anti-*Brucella*, et par l'agglutination d'Osebold (OAT) pour rechercher les anticorps anti-*Listeria*. La séroprévalence de la toxoplasmose a été de 13.5% en utilisant le test IHA et de 28.3% en utilisant le SFDT, ce dernier présentant une meilleure sensibilité. La séroprévalence de la brucellose est restée faible chez les chevaux (9.5%) alors qu'une forte incidence de la listériose (48.6%) a été mise en évidence. Aucune association significative entre le sexe ou l'âge des animaux et l'infection par la toxoplasmose ou la listériose n'a été observée. En revanche, la séroprévalence de la brucellose a été significativement plus élevée chez les femelles que chez les mâles. Ces résultats suggèrent que les chevaux de la région d'Hakkari pourraient constituer des réservoirs infectieux potentiels pour les autres espèces et contribuer à l'extension de ces pathologies et à la contamination de l'homme directement (brucellose, listériose) et indirectement (toxoplasmose).

Mots-clés : Toxoplasmose, Brucellose, Listériose, cheval, Hakkari, séroprévalence.

Introduction

Toxoplasmosis, brucellosis and listeriosis are zoonotic diseases in humans and many other worm-blooded animal species including horses. Toxoplasmosis is caused by *Toxoplasma gondii* which is an obligate intracellular parasite and is able to develop in a wide variety of vertebrate hosts but its definitive hosts are the domestic cats and other *Felidae* [17, 19, 31]. Ingestion of infected meat and food or water contaminated with oocysts is the two major routes of transmission of *Toxoplasma gondii* to humans. There is no definitive evidence of *T. gondii* causing clinical disease in horses [18], but some studies [7, 12] reported clinical symptoms, such as increases of body temperature, ataxia, degeneration of retina and encephalomyelitis, though not specific for disease. *Toxoplasma gondii* together with *Neospora caninum* and *Sarcocystis neurona* are considered to be associated with encephalomyelitis in horses [16, 20]. *Toxoplasma gondii* infection can be detected using serological and histological examinations. Several

serological methods have been used for detecting *T. gondii* antibodies. Sabin –Feldman Dye Test (SFDT), Complement Fixation Test (CFT), Indirect Haemagglutination Test (IHA), Latex Agglutination Test (LAT), Enzyme-linked immunosorbent assay (ELISA) and modified agglutination test are commonly used tests [17, 19, 21, 39].

Brucella is a gram-negative, facultative intracellular bacterium infecting several animal species and human being. Brucellosis is an important zoonotic disease worldwide resulting in heavy economic losses for the livestock industry and causing serious human health problems [11]. *Brucella* is successfully isolated from horses. Brucellosis is generally asymptomatic in horses. But some clinical signs such as fistulous withers, osteoarthritis, osteomyelitis, bursitis and tenosinovitis may develop in some cases. The most obvious brucellosis sign in horses is fistulous withers. Fistulous withers caused by brucellosis are a public health problem. Equine brucellosis can also cause abortion and infertility [8, 10, 15]. For serodiagnosis

of horse brucellosis, many serologic tests such as Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Complement Fixation Test (CFT) and Coombs Test may be used [15, 22, 29].

Listeriosis is a zoonotic disease and infection in animals is caused by *L. monocytogenes*. *L. monocytogenes* is a gram-positive rod, existing in the environment and in the digestive tract of human and animals. Listeriosis can cause septicaemia, gastroenteritis, meningoencephalitis, abortion, mastitis, metritis, conjunctivitis and keratitis. Listeriosis is more common in cattle and sheep but has also been reported in horses [38, 40, 41, 47, 48].

Because these 3 diseases can punctually occur in horses even if they are associated with no specific clinical signs, and because infected horses would constitute infectious reservoirs for other animal species and humans, the aim of this study is to indirectly evaluate the prevalences of toxoplasmosis, brucellosis and listeriosis in horses from the Hakkari Province, in the Eastern Turkey.

Materials and Methods

STUDY AREA AND ANIMALS

Blood samples were collected from horses from the Hakkari province, in the eastern region of Turkey. Hakkari city is on Iranian and Iraq border. Native breed horses aged between 2-6 years in this area are used for short distance transportation. Animal breeding in this area is traditional (cattle, sheep and horses are bred in a same stable). For this reason, many diseases are easily transmitted between animal species.

Approximately 10 ml blood samples were obtained from the jugular vein of 74 local horses (63 males, 11 females) in May 2005 and transported to the Parasitology laboratory. Blood samples were centrifuged at 2 350 g at 4°C for 10 minutes and then serum was removed and stored at -20°C until tested.

SEROLOGICAL ASSAYS

Toxoplasmosis: Sera were tested using Indirect Haemagglutination Test (IHA) (TOXO- HAI, FUMOZE laboratories, France) to determine total serum anti-*Toxoplasma* antibodies. Various dilutions of sera (1:80, 1:160, 1:320, 1:640, 1:1280 and 1:2560) were prepared and

then maintained at room temperature for two hours. Samples in which haemagglutination occurred at 1/160 or higher dilutions were considered positive. Serum samples were also tested for toxoplasmosis by Sabin-Feldman Dye Test (SFDT) using vital antigen and methylene-blue dye. Serum samples were inactivated at 56°C for 30 minutes, then tested for anti *T. gondii* antibodies with fourfold dilutions (1:16, 1:64, 1:256, and 1:1024). An antibody titre of 1/16 or over was accepted to be positive [39].

Brucellosis: Serum samples (diluted to 1/10 to 1/80) were screened for *Brucella* infections by Rose Bengal Test and positive sera were confirmed by Serum Tube agglutination test (SAT). In RBT, 30 µl of serum was mixed with equal volume of antigen on a white enamel plate. The mixture was rocked gently for 4 min at room temperature. Mixtures formed agglutination was considered positive. In SAT, serum samples were diluted at 1:10, 1: 20, 1:40 and 1: 80 with *B. abortus* antigen. The results were evaluated after incubation at 37°C for 12-24 hours [4].

Listeriosis: Osebold Agglutination Test (OAT):

An antibody titration test to detect antibodies for *L. monocytogenes* was carried out according to the method described by OSEBOLD *et al.* [34]. The test antigen used in the present study was prepared in the Laboratories of Refik Saydam National Hygiene Centre, Department of Communicable Diseases Research, and the assay was carried out in 3 steps. For the first step, the whole cell antigens were prepared from *Staphylococcus aureus* (ATCC 29213) strains by the Osebold method. In the second step, *Listeria* antigens were prepared from *L. monocytogenes* 1/2a, 1/2b, 4b, 4c and 4d strains and were combined in the same suspension. In the last step an agglutination test was performed after the absorption of sera samples with *S. aureus* antigen. Samples with a titre 1:100 were considered positive.

STATISTICAL ANALYSES

Statistical analyses (effects of age and sex) were performed with X² test. P values less than 0.05 were considered significant.

Results

Anti *T. gondii* antibodies were found in 10 out of the 74 horses (13.5%) with IHA test. Eight samples were positive with a 1:160 titre and 2, with a 1:320 titre (Table I). On the

Tests	Number of total samples	Number of positive samples (%)	Titres				
			1:160	1:320	1:640	1:280	1:2560
IHA	74	10 (13.5%)	8	2	0	0	0
SFDT	74	21 (28.4%)	1:16	1:64	1:256	1:1024	
			19	2	0	0	

TABLE 1: IHA and SFDT seroprevalences of *T. gondii* in horses (n = 74) from the Hakkari region, Eastern Turkey.

	SFDT – (n = 53)	SFDT + (n = 21)	Sensitivity (Se) False Negative (FN)	Specificity (Sp) False Positive (FP)	Agreement
IHA +	0	10	Se: 47.6%	Sp: 100%	85%
IHA -	53	11	FN: 52.4%	FP: 0%	

TABLE 2: Comparison of IHA test to SFDT of horse (n = 74) antibodies against *Toxoplasma gondii*: relative sensitivity, specificity and agreement.

other hand, 21 horses were positive using SFDT (28.4%) with a 1:16 titre in 19 horses (90.5%) and a 1:64 titre in 2 horses (9.5%) (Table I). Comparison between IHA and SFD test was performed (Table II). Obviously, for a relative specificity of IHA to SFDT of 100% (frequency of IHA negative sera on the total SFDT negative sera) the relative sensitivity of IHA positive sera on the total SFDT positive sera) was low (47.6%) and the corresponding agreement between the 2 methods (number of identical scores) was moderate (85%).

Only seven horses (9.5%) were positive (titre > 1:40) with RBT and presence of anti *B. abortus* antibodies was confirmed by SAT (Table III).

By contrast, a high *L. monocytogenes* seroprevalence (48.6%) was observed in horses from the Hakkari region: 36 sera were positive using OAT and among positive samples, the proportions of high antibody titre (>1:200) was elevated (39%) (Table IV).

In the present study, the effects of age and sex on the seroprevalences of the 3 diseases were also analysed (Table V). Although young animals (< 2 year old) and females appeared to be more frequently infected by toxoplasmosis, no significant interaction of the seroprevalence with age or sex was evidenced. In the same way, males and females were equally affected by the listeriosis and no particular class of ages was predisposed to the infection. By contrast, females were significantly more frequently infected by *Brucella* than males ($p < 0.01$).

As shown in Table VI, out of the 51 horses positive for toxoplasmosis, brucellosis or listeriosis, 13 animals (25.5%) were simultaneously infected by 2 micro-organisms, particularly by *T. gondii* and *L. monocytogenes* (69.2% of the poly-infected horses). Furthermore, 57.1% positive sera for toxoplasmosis (12/21) or for brucellosis (4/7) were also positive for other infectious agents. Nevertheless, no significant association between the infectious agents was evidenced. No case of triple infection was observed.

Number of total samples	Number of positive samples (%)	Titres	
		1:40	1:80
74	7 (9.5 %)	5	2

TABLE 3: SAT seroprevalence of *B. abortus* in horses (n = 74) from the Hakkari region, Eastern Turkey.

Number of total samples	Number of positive samples (%)	Titres		
		1:100	1:200	1:400
74	36 (48.6 %)	22	13	1

TABLE 4: OAT seroprevalence of *L. monocytogenes* in horses (n = 74) from the Hakkari region, Eastern Turkey.

	Number of Toxoplasmosis positive sera	Number of Brucellosis positive sera	Number of Listeriosis positive sera
Classes of age (in year)			
[0; 2[(n = 26)	11	3	8
[2; 5[(n = 28)	6	4	16
> 5 (n = 20)	4	0	12
Sex			
Males (n = 63)	16	3**	32
Females (n = 11)	5	4**	4

** $p < 0.01$

TABLE 5: Seroprevalence of toxoplasmosis (using SFDT), of brucellosis and of listeriosis in horses (n = 74) from the Hakkari region, Eastern Turkey according to the age and sex.

Infectious agent	Positive horses		Total
	Mono-infected	Poly-infected	
<i>Toxoplasma gondii</i>	9	12 (<i>Brucella</i> + : 3 / <i>Listeria</i> +: 1)	21
<i>Brucella abortus</i>	3	4 (<i>Toxoplasma</i> +: 3 / <i>Listeria</i> +: 1)	7
<i>Listeria monocytogenes</i>	26	10 (<i>Toxoplasma</i> +: 9 / <i>Brucella</i> + : 1)	36
Total	38	13	51

TABLE 6: Cases of poly-infections with toxoplasmosis, brucellosis or listeriosis in horses (n = 74) from the Hakkari region, Turkey, serologically detected by SFDT, RBT and OAT respectively.

Discussion

Toxoplasmosis, brucellosis and listeriosis are diseases that have zoonotic potential in horses [17, 18, 31]. In Turkey, toxoplasmosis in horses was first reported by WEILLAND and DALCHOW in 1970 [46]. These authors have reported that seroprevalence of the *T. gondii* using SFDT was found 14.3% out of the investigated 154 horses. Many studies on toxoplasmosis in horses were performed using SFDT and other serodiagnosis tests in different region of Turkey after 1996. Using SFD test, the reported *T. gondii* seroprevalences ranged from 1.8% to 20.6% according to the different regions of Turkey ([49], Kars [1, 4], Bursa [23], Ankara [5, 6], Kayseri [24], Southern Anatolia [35] and Malatya [3] regions). Despite the great heterogeneity of these previous results, no particular region seemed mainly infected by *T. gondii*, and particularly no province close to the Hakkari region. Compared to the SFDT, the toxoplasmosis seroprevalence determined with IHA test will be under-estimated because of the lower sensitivity of this test. Furthermore, in a previous study conducted in 172 horses from the Van city in the Eastern city, Turkey, the reported prevalence of anti *T. gondii* antibodies using IHA was 1.75% [2]. In our study, the seroprevalence of Toxoplasmosis in the Hakkari city bordering the Van city was 13.5% using the same test and was 28.4% with SFDT. Only some technical aspects (different commercial diagnostic kits or different serial dilutions) would explain such discrepancies between the results obtained in these 2 closely located cities.

The determination of anti *T. gondii* antibodies in horses does not imply a direct risk for human health because horse meat is not served for human consumption in Turkey. But toxoplasmosis occurrence in horses would favour the infection spreading; indeed, horse carcasses are used for carnivore food and dead horses left outside in villages were eaten by stray dogs and cats. This practice may contribute to the disease transmission between animal species and indirectly to human. This finding may suggest further study involving other animal species.

Brucellosis, a zoonotic disease, inflicts significant economical losses to agricultural industry and is also the most important threat to human health [11]. Infection of horses by *Brucella abortus* is a long known fact. Although isolation of *Brucella abortus* from horses in Turkey has not yet been reported, studies revealed its isolation from horses in other parts of the world [32]. A few studies have been performed on prevalence of brucellosis in horses in other countries. Seroprevalence of brucellosis in horses reported to be between

0-12.8% using SAT method in these countries [15, 22, 29, 33, 42]. Serological tests are commonly used to diagnose of brucellosis in horses. The most reliable test is Serum Tube Agglutination (SAT) and that titre equal or greater than 1:40 is accepted as positive for horses [15]. Our study revealed that 7 of 74 horses examined had titres greater than 1:40. This high antibody titre is the first reported for horses in Turkey [13, 25, 44]. The finding of remaining horses (n = 67) having titre between 1:10-1:20 may be related to the traditionally husbandry methods where cattle, sheep and horses are housed together. The extension of brucellosis among sheep and horses may not only be a source of infection for other animals but also for human beings. This is important since traditional animal husbandry practices encourages herding of animals. Therefore, the disease may spread easily to man and to other animals. Equines are possibly shedders of *Brucella* and may be a potential source of infection for other animals and man [32, 36]. In our study, the higher rate of brucellosis seroprevalence was detected in females in comparison to males. Although no particular susceptibility of female horses for brucellosis was mentioned in the literature, the seroprevalence of brucellosis was detected two-folds higher in females compared to males in camels [36]. In another study performed in cattle and buffaloes, seroprevalences of brucellosis detected in males and in females were 3.6 % and 4.9 % respectively [43].

Listeriosis is also a zoonotic disease of man and domestic animals including horses [24]. *Listeria monocytogenes* has been implicated that cause of food borne outbreaks in recent years [27, 28]. A variety of animals including domestic farm animals can carry *Listeria* species and are considered potential vectors of this organism. Horses can also be sources of *Listeria* species [9]. Contamination of the soil and water sources with *Listeria* spp. may occur through faeces by all the animals including horses. *Listeria* spp. and *Listeria monocytogenes* has been detected in horse meat for human consumption [14, 30]. But this contamination route should not be the case in this country, because it is not consumed by men.

Only scarce studies on listeriosis were conducted in horses from Turkey [24, 26, 41, 44]. An outbreak of listeriosis in 200 pregnant mares was reported in 1945 when 12 encephalitic cases were diagnosed and 5 of these died [41]. INCI *et al.* [24] carried out a study on listeriosis in horses in Kayseri using Osebold test and found 27 positive of 67 horses (40.3%). SOLMAZ *et al.* [44] undertook a similar study in Van using Regnault's Tube Agglutination (RTA) method [37] and reported that 176 horses of 203 (86.69%) were seropositive.

In our study carried out on horses in Hakkari city bordered by Iran and Iraq using Osebold method 48.5% of 74 examined horses were seropositive. This finding was in agreement with the INCI's study [24] but the observed seroprevalence was markedly lower than the results obtained with the same method but lower than the figure by SOLMAZ *et al.* [44], probably because of the highest sensitivity of the RTA.

In conclusion, this was the first and preliminary study of its kind where three major zoonotic diseases were investigated in horses in Hakkari and anti-*brucella* antibody titres greater than 1:40 was first reported in horses in Turkey. These findings imply that further detailed studies are needed in the region not only for economical reasons but also for human and animal health.

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